PB85-198307

Toxicity of Ammonia to Early Life Stages of the Smallmouth Bass at Four pH Values

(U.S.) Environmental Research Lab.-Duluth, MN

1985

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before complet

1. REPORT NO. EPA/600/J-85/032	2.	3. PB85-198307
4. TITLE AND SUBTITLE TOXICITY OF AMMONIA TO EARLY LIFE STAGES OF THE SMALLMOUTH BASS AT FOUR pH LEVELS (Journal Version)		5. REPORT DATE 1985 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) S. Broderius, R. Drummond, J. Fiandt, and C. Russom		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth 6201 Congdon Boulevard Duluth, MN 55804		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Duluth, MN 55804		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/03

15. SUPPLEMENTARY NOTES

Environ. Toxicol. Chem. 4: 87-96 (1985)

16. ABSTRACT Early life stages of smallmouth bass (Micropterus dolomieui Lacepede) were exposed to ammonia concentrations at four different pH values (from 6.5 to 8.7). Acute lethal tests were conducted with juveniles, and partial chronic tests were initiated at the late embryo stage and extended through 32 d of exposure.

The 96-h LC50 ammonia concentrations at pH 6.53, 7.16, 7.74 and 8.71 were 371, 117, 39.5 and 7.43 mg/l total ammonia-N (NH_3-N) and 0.694, 1.00, 1.20 and 1.78 mg/l un-ionized NH_3, respectively. The acute toxicity of ammonia in terms of NH_3-N was directly related to test pH, and in terms of NH_3 was inversely related to pH.

Embryo through preswim-up larvae were the most resistant early life stages studied during chronic exposures. Survival of embryos to larvae 7 d post hatch was unaffected by ammonia concentrations ranging from 0.236 to 0.865 mg/l NH_3 at pH values of 6.60 to 8.68. Adverse effects on early developmental stages demonstrated that survival to 14 d and growth to 32 d were among the most sensitive indicators of ammonia toxicity. The estimated 32-d no-observed-effect concentrations, defined as the geometric means of the maximum no-effect and minimum effect concentrations determined from statistically significant decreases in growth (wet weight), were calculated to be 17.4, 14.4, 14.6 and 2.40 mg/l total NH_3-N and 0.0437, 0.148, 0.599 and 0.612 mg/l un-ionized NH_3 at pH values of 6.60, 7.25, 7.83 and 8.68, respectively. The acute to chronic ratios, as calculated from NH_3 concentrations and acute results adjusted to the above pH values for chronic tests, were 16.7, 7.03, 2.07 and 2.89, respectively.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 11
	) SECURITY CLASS *(This page)* Unclassified	22. PRICE

REPRODUCED BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA. 22161

Environmental Toxicology and Chemistry, Vol. 4, pp. 87–96, 1985
Printed in the USA. Pergamon Press Ltd.

0730-7268/85 $3.00 + .00
1985 SETAC

EPA/600/J-85/032
JOURNAL ARTICLE

TOXICITY OF AMMONIA TO EARLY LIFE STAGES OF THE SMALLMOUTH BASS AT FOUR pH VALUES

STEVEN BRODERIUS,* ROBERT DRUMMOND, JAMES FIANDT and CHRISTINE RUSSOM
Environmental Research Laboratory–Duluth Office of Research and Development, U.S. Environmental Protection Agency, Duluth, Minnesota 55804

(*Received* 5 *December* 1983; *Accepted* 27 *April* 1984)

Abstract—Early life stages of smallmouth bass (*Micropterus dolomieui* Lacepede) were exposed to ammonia concentrations at four different pH values (from 6.5 to 8.7). Acute lethal tests were conducted with juveniles, and partial chronic tests were initiated at the late embryo stage and extended through 32 d of exposure.

The 96-h LC50 ammonia concentrations at pH 6.53, 7.16, 7.74 and 8.71 were 371, 117, 39.5 and 7.43 mg/L total ammonia-N (NH_3-N) and 0.694, 1.00, 1.20 and 1.78 mg/L un-ionized NH_3, respectively. The acute toxicity of ammonia in terms of NH_3-N was directly related to test pH, and in terms of NH_3 was inversely related to pH.

Embryo through preswim-up larvae were the most resistant early life stages studied during chronic exposures. Survival of embryos to larvae 7 d post hatch was unaffected by ammonia concentrations ranging from 0.236 to 0.865 mg/L NH_3 at pH values of 6.60 to 8.68. Adverse effects on early developmental stages demonstrated that survival to 14 d and growth to 32 d were among the most sensitive indicators of ammonia toxicity. The estimated 32-d no-observed-effect concentrations, defined as the geometric means of the maximum no-effect and minimum effect concentrations determined from statistically significant decreases in growth (wet weight), were calculated to be 17.4, 14.4, 14.6 and 2.40 mg/L total NH_3-N and 0.0437, 0.148, 0.599 and 0.612 mg/L un-ionized NH_3 at pH values of 6.60, 7.25, 7.83 and 8.68, respectively. The acute to chronic ratios, as calculated from NH_3 concentrations and acute results adjusted to the above pH values for chronic tests, were 16.7, 7.03, 2.07 and 2.89, respectively.

Keywords—Ammonia Toxicity pH Fish Smallmouth bass

INTRODUCTION

Ammonia is a commonly occurring chemical that has toxic effects at low concentrations on fishes and other aquatic organisms. Its presence in natural waters arises primarily from sewage effluents, industrial wastes and agricultural runoff.

In aqueous solution, ammonia is present in gaseous or un-ionized (NH_3) and ionized (NH_4^+) forms. The percentage of total ammonia present in the un-ionized form and its toxicity to freshwater organisms is markedly affected by pH and temperature [1]. As pH and temperature increase, the ammonia equilibrium is shifted toward the NH_3 chemical species [2]. Un-ionized ammonia has long been thought to be the primary toxic form responsible for the effects of ammonia on aquatic life. Some authors have suggested that the ionized form of ammonia may also contribute to observed ammonia toxicity [1].

A review of the toxicity of ammonia to aquatic organisms [3–5] reveals a considerable amount of information on the sensitivity of coldwater fishes and inadequate knowledge on the lethal and sublethal effects of ammonia on sensitive life stages of warmwater fishes. Previous toxicological studies with ammonia and involving freshwater bass have been limited to one report on acute toxicity [6]. Two recent studies [7; W. E. Smith and T. H. Roush, manuscript in preparation] reported on both the acute and chronic effects of ammonia on other members of the Centrarchidae family.

The objectives of this study were to determine the lethal and sublethal effects of ammonia on early life stages of the smallmouth bass at four different pH values, from 6.5 to 8.7. Two types of tests were conducted: (a) standard 96-h acute tests with juveniles and (b) early life stage (ELS) tests

*To whom correspondence may be addressed.

starting with 2- to 3-d-old embryos and lasting through 32 d post hatch.

MATERIALS AND METHODS

Test species

Smallmouth bass (*Micropterus dolomieui* Lacepede) embryos were obtained from three recent natural spawnings in an outdoor pond at the New London Fish Hatchery, U.S. Fish and Wildlife Service, New London, Minnesota. Juvenile fish used in the acute toxicity tests were reared from the same lot of embryos used to start the ELS chronic tests. These fish were cultured in Lake Superior water at 22 ± 1°C. At least twice daily during the rearing period they were fed recently hatched brine shrimp nauplii (*Artemia* sp.), hatched in the laboratory from eggs (San Francisco Bay Brand; Metaframe, Inc.), and frozen adult brine shrimp (Jungle Laboratories Corp., Sanford, FL) during the later portion of the rearing period.

Dilution water

The dilution water was taken directly from Lake Superior. The water was passed through a sand and gravel filter, then a 50 μm cotton fiber filter, and delivered through polyvinylchloride pipes to stainless steel head tanks. Characteristics of the dilution water other than those measured were assumed to be similar to that previously reported [8].

Dilution water to the test chambers was supplied from two 25-liter stainless steel head tanks. Temperature was controlled in each head tank by a mercury switch in conjunction with electric heating elements. The water in each head tank was pumped over a vertical and cylindrical nylon screen to remove excess dissolved gases. The pH of the dilution water in one tank was not adjusted (pH 8.0) while that in a second tank was maintained at about 6.3 by continuous addition of dilute hydrochloric acid and removal of excess CO_2 by mechanical aeration. By mixing water from both head tanks with glass–Teflon mixing valves, a third test water of pH 7.25 was obtained. By adding a dilute solution of sodium hydroxide directly to the ambient head tank water (pH 8.0) just before it entered the diluter, a fourth dilution water of pH 8.75 was provided. Average pH was determined by converting pH values to their corresponding hydrogen ion concentrations, averaging these values, and then reconverting the averages to pH units.

Exposure system

The acute and partial chronic tests were conducted in two identical diluters with continuous-flow water delivery and toxicant introduction systems similar to that previously described [9]. These units provided the necessary test conditions, with two controls and six duplicated ammonia concentrations in a geometric series (0.6 dilution factor) at each of four pH values.

The test chambers were constructed of glass and silicone sealant. Each chamber measured 20.4 × 10.2 × 10.2 cm high, with an overflow outlet at a height of 5 cm; each contained a volume of 1 liter. Dilution water flow rate to each test chamber was 40 ml/min, affording 99% replacement in about 2 h [10]. Test aquaria were placed in a random arrangement for each pH series. The order for duplicate tanks, however, was the same at each pH level, with the A and B tanks kept in distinct blocks.

Egg incubation cups, made from 4-oz, 5-cm o.d. round glass jars with the bottoms cut off and replaced with 40-mesh stainless steel screen, were vertically oscillated about 1 inch in the test water by means of a rocker arm apparatus driven by a 2-rpm motor [11]. The fluctuating water level ensured water circulation around the embryos.

A 16-h photoperiod was maintained, including a 30-min gradual brightening and dimming period to simulate dawn and dusk [12]. A combination of incandescent and fluorescent bulbs was used in the laboratory, with wide-spectrum 40-W 4-ft fluorescent bulbs (Durotest "Vitalite") 14 cm above the test chambers. The light intensity at the test water surface ranged from 22 to 38 lumens per square foot.

Certified ACS-grade ammonium chloride (lot 716942; Fisher Scientific Co.) was used as the toxicant. Stock solutions were prepared in distilled water and delivered to the diluters by FMI metering pumps (Fluid Metering, Inc., Oyster Bay, NY). The pH of each stock solution was adjusted to that of the desired test pH with a 15 N sodium hydroxide solution.

Analytical procedures

Toxicity modifying factors such as pH, dissolved oxygen, hardness and alkalinity were frequently measured in the water from control and treatment chambers according to standard analytical procedures [13].

Total ammonia nitrogen (NH_3-N) concentrations were determined by the direct Nesslerization

Method (method 417B) [13]. Spectrophotometric measurements were made with a Beckman DB-G double-beam spectrophotometer equipped with digital display. Un-ionized ammonia (NH_3) concentrations were calculated [2,14] from measured total NH_3-N, test pH and temperature. The pH and temperature in each test treatment were determined at approximately the same time as the samples were taken for ammonia determinations. Test solutions were sampled directly from test chambers and analyzed according to a monitoring program that adequately characterized the exposure pattern. All total ammonia concentrations were measured daily for acute tests and three times a week for chronic tests in water from alternate replicate exposure chambers at each treatment level. Determinations for each test treatment were averaged and used as the experimental exposure parameters. The concentration of total NH_3-N in the control tanks was about equal to or below the detection limit of 0.10 mg/L.

Duplicate measurements of total ammonia were routinely made with each analytical series to define the reproducibility of the measurements. Comparisons of chemical concentrations between duplicate exposure tanks sampled simultaneously and at the highest treatment level showed that concentrations for 16 acute and 48 chronic exposure determinations were within $99.9 \pm 2.12\%$ and $100 \pm 2.41\%$ of each other, respectively. Spiked recoveries for 16 and 50 measurements during acute and chronic tests were $103 \pm 3.20\%$ and $103 \pm 6.66\%$, respectively.

Nitrate and nitrite nitrogen concentrations were measured (methods 418A and 419) [13] once and twice weekly, respectively, during chronic tests. These nitrogen determinations were made once, on the third day, during each 4-d acute test.

Acute test procedure

The method for acute tests in general followed standard procedures [15]. The test exposure systems were operated without toxicant addition or pH control for about 1 week before the test fish were added. Ten smallmouth bass were then randomly added, five at a time to each treatment and control chamber, thus providing a total of 20 juveniles per treatment level. The fish were not fed for 24 h before or during the acute toxicity tests. Age of the fish used in acute tests was approximately 40 d post hatch at the start of the tests. Overall mean total length and wet weight (with ranges) for the juvenile smallmouth bass in the controls were 29 (26–35) mm and 265 (203–353) mg, respectively. The corresponding ranges in mean values for the four individual test control groups were 29–29 mm (total length) and 264–267 mg (wet weight).

After 2 h of acclimation by the test fish to the exposure systems, pH control was initiated. Once the desired pH at all four levels was attained (about 4 h after initiating pH control), ammonia was added to achieve the desired equilibrium ammonia concentration in each test chamber. Therefore, about 6 h should have elapsed from initiation of pH control to attainment of equilibrium conditions. It is possible that this short acclimation time may have compromised our acute results, especially at the lowest pH level, but this is doubtful since no inexplicable results were observed.

Mortalities were recorded daily, and estimated LC50 values and 95% confidence intervals were determined by the Trimmed Spearman-Karber method [16]. These values were calculated for each test from both the average total NH_3-N and NH_3 concentrations in each test chamber.

Chronic test procedure

The method for chronic tests in general followed standard procedures [17]. The sublethal tests were begun with smallmouth bass in the late embryo to early eleutheroembryonic phase of development [18]. The tests were initiated by adding five embryos at a time to 56 egg baskets until a total of 50 in each was attained. The embryos were examined with the aid of a dissecting microscope (10×) so that only normal-appearing individuals of comparable development were selected. These baskets were then randomly transferred to each duplicate test chamber. Immediately after adding the baskets to the test chambers, pH control was initiated. Once the four test pH values were attained (about 4 h after initiating pH control), ammonia was added to achieve the desired concentration in each test chamber.

Mortality, as determined under a dissecting microscope, was recorded daily. Dead embryos were removed, to prevent the spread of fungus growth, when their contents began to turn an opaque white. Fry mortality was determined by no detectable heart beat and an opaque white coloration. The hatching process was nearly completed at the time the test was started. At the time of swim-up and initial feeding (7 d into the test) the surviving smallmouth fry were transferred from the egg baskets into each respective test chamber.

Two weeks after initiation of the test, the numbers of surviving larvae in each test chamber were impartially reduced to no more than 20 each. This more nearly equalized the number of individuals in all chambers. The thinning was done to prevent crowding and provide more uniform growth conditions.

Beginning at the time of swim-up, fry were fed *ad libitum* but equal volumes of newly hatched brine shrimp (*Artemia* sp.) nauplii three times daily. The brine shrimp, in a well-mixed freshwater slurry, were dispensed to each chamber with an open-bore medicine dropper. At treatment levels at which the survival of fish was affected, the volumes were roughly proportional to the number of survivors. This helped to ensure that growth effects were a result of the treatment levels and not due to disproportionate food quantities. The test aquaria were siphoned daily to remove fecal material, detritus and bacterial and fungal growths.

The tests were terminated after 32 d of exposure by sacrificing the fish by immersion in ice water. The fish were last fed 24 h before test termination. Individual total lengths to the nearest millimeter and blotted-dry wet weights to the nearest milligram were determined for the surviving juveniles.

The effects of various treatment levels were statistically analyzed [19]. Individual lengths and wet weights were transformed to logarithms and percentage survival data to arc sine square root of survival proportions. Lengths, wet weights and survival data were subjected to one-way analysis of variance (ANOVA) with an F test. Treatment means were compared with control means using Dunnett's pairwise comparison procedure (one-tailed) at a significance level of 1.0 or 5.0%. Using this procedure it was statistically possible to determine which of the treatment means differed significantly from a control mean.

A chronic no-observed-effect concentration (or maximum acceptable toxicant concentration) was calculated from the geometric mean of the maximum no-effect concentration and the minimum effect concentration. An acute-to-chronic ratio was calculated by dividing the 96-h LC50 concentration by the chronic no-observed-effect concentration when both endpoints were expressed as NH_3 and acute results were adjusted to that expected at the pH of chronic tests. The normalization of acute results was accomplished by first defining the relationship between test pH and acute toxicity of NH_3, with a linear regression equation (96-h LC50 mg/L $NH_3 = -2.54 + 0.492$ pH, $r = 0.995$). Using only the slope of this equation, the acute results were adjusted so that corrected values represented the acute response expected if acute tests had been conducted at exactly the same pH as in the chronic tests. This correction amounted to a maximum increase of 4.9% with a pH increase of 6.53 to 6.60, and to a decrease of 0.56% with a pH decrease of 8.71 to 8.68.

RESULTS AND DISCUSSION

Acute tests

The overall mean values for certain physical and chemical characteristics of the test water were calculated because measurements for these parameters did not differ much among treatment levels for the four acute or early life stage toxicity tests. The overall mean (±1 SD) and range of separate values for these parameters during the four acute toxicity tests were as follows: temperature, in °C, 22.3 ± 0.05 (21.8–23.2); dissolved oxygen, in mg/L, 7.95 ± 0.24 (7.70–8.30); hardness, as mg/L $CaCO_3$, 42.3 ± 0.89 (40.7–43.7); nitrate, as mg/L NO_3-N, 0.28 ± 0.006 (0.27–0.29); and nitrite, as μg/L NO_2-N, 0.47 ± 0.14 (0.39–0.70). The average pH and alkalinity measurements from all tanks for each acute test are presented in Table 1. Temperature and pH have a direct controlling influence on aqueous ammonia equilibrium, and alkalinity varied directly with test pH because of the way in which pH was controlled. The maximum free carbon dioxide concentration was calculated [13] to be about 5 mg/L at test pH 6.53.

No mortality was observed in any control tanks. The acute toxicity values for the 96-h tests are reported (Table 1) in terms of both NH_3 and total NH_3-N. It is apparent that the toxicity of NH_3 and total NH_3-N are indirectly related to one another as test pH varies. The toxicity of NH_3 to smallmouth bass does not remain constant over a wide pH range, since NH_3 LC50 values are considerably lower at low compared with high pH values. At least three explanations can be offered for this observation: The simplest is that the toxicity of NH_3 is not constant over the pH range tested and, in fact, that it increases with decreasing pH. A second explanation might be that NH_4^+ in the test solution exerts a toxic effect through active and/or passive uptake by the test fish. This is especially true in low test pH solutions, in which the LC50 concentrations of total ammonia are relatively high. With regard to the latter explana-

Table 1. Acute toxicity of ammonia to juvenile smallmouth bass at different pH values[a]

pH	Alkalinity (mg/L as $CaCO_3$)	96-h LC50 (95% CI)[b] Un-ionized NH_3 (mg/L)	96-h LC50 (95% CI)[b] Total Ammonia-N (mg/L)
6.53 ± 0.12 (6.34–6.69)	9.15 ± 0.48 (8.87–9.85)	0.694 (0.497–0.936)[c]	371 (267–498)[c]
7.16 ± 0.04 (7.08–7.24)	25.9 ± 1.60 (23.6–28.6)	1.00 (0.933–1.08)	117 (109–125)
7.74 ± 0.02 (7.71–7.79)	45.8 ± 2.90 (43.3–51.2)	1.20 (1.08–1.34)	39.5 (35.4–44.2)
8.71 ± 0.04 (8.61–8.76)	52.2 ± 5.66 (46.3–61.1)	1.78 (1.69–1.87)	7.43 (7.09–7.77)

[a]Data presented for pH and alkalinity measurements from all tanks for each test are mean values ±1 SD, with ranges in parentheses.
[b]CI, confidence interval.
[c]95% CI could not be reliably calculated; reported values represent the concentrations at which 0 and 95% mortality, respectively, was observed.

tion, it has been estimated [1] that, on a molar comparison basis, NH_3 is 300 to 400 times more toxic than NH_4^+ to rainbow trout (*Salmo gairdneri*) and the fathead minnow (*Pimephales promelas*). After conducting similar calculations [20], we estimate this toxicity ratio for molecular versus ionized ammonia from acute tests with smallmouth bass to be about 150. It is our opinion that a third explanation involving a pH drop at the gill surface [21,22] is not a valid interpretation of the observed change in ammonia toxicity. This was also the conclusion reached in interpreting the observed change with test pH in toxicity of hydrogen sulfide and hydrogen cyanide on the fathead minnow (*P. promelas*) [23]. Therefore, it appears that no definite explanation can be given for the changes in toxicity of ammonia to freshwater fish that occur with changes in test pH.

No data could be found in the published literature on the acute toxicity of ammonia to smallmouth bass. Our 96-h LC50 values of 1.20 and 1.78 mg/L NH_3, as determined at pH values of 7.74 and 8.71, respectively, and for smallmouth bass of 265 mg, are in good agreement with values reported for largemouth bass [6]. The reported 96-h LC50 values of 1.0 and 1.7 mg/L NH_3 are for tests conducted with bass ranging in size from 0.09 to 6.3 g and at pH levels similar to those in our tests. The acute toxicity of ammonia for juvenile smallmouth bass in our study is also within the range of toxicity values reported in the literature for other warmwater fish species of the Centrarchidae family [5]. This is particularly true for tests conducted at comparable pH levels and with fish of similar size.

Chronic tests

The overall mean (±1 SD) and range of separate values for certain physical and chemical characteristics of the test water during the four early life stage tests were as follows: temperature, in °C, 22.3 ± 0.25 (21.7–23.0); dissolved oxygen, in mg/L, 7.72 ± 0.39 (7.10–8.30); hardness, as mg/L $CaCO_3$, 43.1 ± 0.77 (41.7–45.6); and nitrate, as mg/L NO_3-N, 0.28 ± 0.03 (0.20–0.33). The average pH, alkalinity and nitrite measurements from all tanks for each early life stage test are presented in Table 2. The good control of the environmental conditions influencing aqueous ammonia equilibrium and its toxicity is apparent. As observed in the acute tests, alkalinity varied directly with test pH. The maximum free carbon dioxide concentration was calculated [13] to be about 4 mg/L at test pH 6.60.

Nitrification of ammonia was more apparent in the long-term tests than during the 4-d acute tests. The formation of NO_2-N was directly related to ammonia concentration and test duration. The optimum pH for nitrite formation was near 7.8, with a decrease in nitrification occurring at both lower and higher pH values. Concentrations over 100 μg/L NO_2-N were not observed at any test level until during the last week of the experiment. Although the few high nitrite concentrations,

Table 2. Physical and chemical characteristics of the test water during early life stage tests[a]

pH	Alkalinity (mg/L as $CaCO_3$)	Nitrite (μg/L NO_2-N)
6.60 ± 0.08 (6.48–6.97)	8.33 ± 0.92 (5.91–10.0)	4.38 ± 7.61 (0.24–12.3)
7.25 ± 0.06 (7.15–7.71)	24.4 ± 2.54 (19.0–27.6)	12.8 ± 22.9 (0.24–114)
7.83 ± 0.09 (7.65–8.06)	42.8 ± 1.56 (39.4–46.0)	22.0 ± 45.0 (0.47–172)
8.68 ± 0.10 (8.46–8.92)	47.0 ± 2.21 (42.3–53.0)	11.2 ± 23.1 (0.47–108)

[a]Data from all tanks for each test are mean values ±1 SD, with ranges in parentheses.

which occurred only at high ammonia concentrations, were undesirable [24], it is believed that these levels did not result in undue stress influencing the observed long-term effects of ammonia on smallmouth bass. The concentrations of NO_3-N formed were not considered to affect test results.

The percentage survival and growth in length and weight of smallmouth bass after exposure to ammonia at each of four pH values are presented in Table 3a–d. It is proposed that different groups of fish at different pH values received similar amounts of brine shrimp, since there was no significant difference in growth among the four controls ($p < 0.05$). Mean weights of 32-d-old control fish ranged from 201 to 206 mg.

Survival of larval fish for the first 7 d and presence of deformed fish throughout the test period were not sensitive indicators of ammonia toxicity. Similar results were reported for pink salmon (*Oncorhynchus gorbuscha*) [25], rainbow trout (*S. gairdneri*) [26] and green sunfish (*Lepomis cyanellus*) [7]. Survival to 7-d-old larvae was virtually unaffected at all ammonia concentrations for three of the four tests. The only exception was for the highest ammonia concentration at pH 8.68, at which complete mortality occurred within 7 d (Table 3d). Daily counts of live fish showed that the greatest reduction in numbers of test fish occurred within the initial 2 weeks. During this period larval fish must make the critical transition from endogenous to exogenous feeding. Lack of an optimum food may have contributed to this reduced survival, but it was assumed that fish in all exposures were affected similarly. The adverse effect, however, was directly related to the ammonia concentration. Overall mean survival of control fish for the first 2 weeks ranged from 62 to 83%. Survival thereafter was nearly 100% for all four controls and at treatment levels that had no other observed adverse effects.

The growth of smallmouth bass at low ammonia concentrations was very similar to that of the controls. We did not observe growth enhancement at low ammonia concentrations, as reported for chinook salmon (*O. tschawytscha*) [27] and green sunfish (*L. cyanellus*) [7]. Growth of juvenile fish to 32 d was among the most consistent and sensitive indicators of toxic stress due to ammonia during the four early life stage tests. The growth in both length and wet weight were about equal in defining statistically significant adverse effects. The estimated chronic no-observed-effect levels for smallmouth bass exposed to ammonia can be determined from results presented in Table 3a–d. The geometric means of the maximum no-effect concentration and minimum effect concentration, as determined from a statistically significant reduction in final mean wet weight as compared with the controls, for total NH_3-N and NH_3 in mg/L, respectively, are as follows: at pH 6.60, 17.4 and 0.0437; at pH 7.25, 14.4 and 0.148; at pH 7.83, 14.6 and 0.599; and at pH 8.68, 2.40 and 0.612. A summary of the acute and chronic toxicity to smallmouth bass of total NH_3-N and NH_3 within the pH range of 6.5 to 8.7 is presented in Figure 1.

Using the acute and chronic no-observed-effect NH_3 concentrations, acute-to-chronic ratios for acute values, corrected to the pH of chronic tests, were as follows: at pH 6.60, 16.7; at pH 7.25, 7.03; at pH 7.83, 2.07; and at pH 8.68, 2.89. The decline in acute-to-chronic ratio with increase in pH is approximately linear between pH 6.6 and 7.8. Beyond pH 7.8 the ratio was essentially independent of pH, to a pH value of about 8.7. This change in the acute-to-chronic ratio reveals the inadequacy of determining a ratio at one pH and applying it to another pH level. Therefore, in using acute-to-chronic ratios to estimate safe concentrations of ammonia, caution should be used in making these calculations at other than tested conditions.

Acknowledgements—The authors wish to thank Charles Gernes of the U.S. Fish and Wildlife Service, New London Fish Hatchery, New London, Minnesota, for the bass eggs and Lawrence Herman and J. Howard McCormick who collected them. We are grateful to Donald Ruppe for analytical chemical assistance. Our sincere appreciation is also extended to Dr. Richard Anderson, the ERL-Duluth data processing and statistical support group and the editorial review group.

Table 3a. Survival, length and weight of smallmouth bass after exposure to ammonia at pH 6.60[a]

Response	Total N: NH_3:	Ammonia concentration (mg/L)						
		117 ± 10.2 0.236 ± 0.0437	61.0 ± 4.82 0.155 ± 0.0506	38.1 ± 3.44 0.0943 ± 0.0324	23.2 ± 3.49 0.0558 ± 0.0180	13.0 ± 0.947 0.0342 ± 0.0132	8.24 ± 0.703 0.0215 ± 0.0096	Control
Survival of 7-d-old sac fry (%)[b]		80 (74–86)	86 (84–88)	87 (84–90)	78 (76–80)	82 (80–84)	88 (86–90)	85 (82–88)
Survival of 14-d-old fry (%)[b]		0** (0–0)	31** (18–44)	36** (36–36)	40* (28–52)	57 (48–66)	77 (74–80)	83 (78–88)
Survival of 32-d-old fish (%)[c]		–	59** (56–60)	89 (78–100)	97 (95–100)	100 (100–100)	90 (85–95)	100 (100–100)
Mean total length of 32-d-old fish (mm)		–	21.8 ± 2.70** (21.8–21.8)	23.8 ± 1.66** (23.2–24.6)	24.6 ± 1.44** (24.4–24.9)	25.2 ± 1.18* (25.2–25.3)	25.7 ± 1.30 (25.4–26.0)	26.1 ± 1.23 (26.0–26.2)
Mean wet weight of 32-d-old fish (mg)		–	139 ± 49.3** (136–148)	161 ± 32.9** (145–182)	177 ± 31.1** (176–178)	187 ± 23.5 (183–191)	199 ± 27.4 (199–200)	201 ± 23.9 (198–204)

Table 3b. Survival, length and weight of smallmouth bass after exposure to ammonia at pH 7.25[a]

Response	Total N: NH_3:	Ammonia concentration (mg/L)						
		51.5 ± 2.68 0.480 ± 0.0569	31.5 ± 1.89 0.303 ± 0.0515	18.4 ± 2.37 0.182 ± 0.0417	11.2 ± 0.987 0.120 ± 0.0311	6.58 ± 0.262 0.0684 ± 0.0159	4.20 ± 0.326 0.0442 ± 0.0105	Control
Survival of 7-d-old sac fry (%)[b]		82 (82–82)	80 (80–80)	85 (84–86)	80 (74–86)	85 (82–88)	80 (78–82)	87 (82–92)
Survival of 14-d-old fry (%)[b]		41* (34–48)	50* (36–64)	56 (54–58)	51* (40–62)	72 (68–76)	62 (56–68)	83 (82–84)
Survival of 32-d-old fish (%)[c]		78* (65–90)	100 (100–100)	100 (100–100)	98 (95–100)	98 (95–100)	98 (95–100)	98 (95–100)
Mean total length of 32-d-old fish (mm)		22.5 ± 2.54** (21.4–24.4)	24.3 ± 2.10** (23.6–24.9)	25.0 ± 1.48 (24.3–25.6)	25.5 ± 1.45 (25.1–25.8)	25.4 ± 0.96 (25.4–25.4)	26.3 ± 1.26 (26.2–26.5)	25.7 ± 1.85 (25.2–26.2)
Mean wet weight of 32-d-old fish (mg)		145 ± 48.7** (123–181)	171 ± 47.0** (163–179)	183 ± 27.9* (179–187)	194 ± 30.4 (192–196)	196 ± 20.6 (193–200)	210 ± 26.7 (208–213)	201 ± 32.9 (199–203)

[a]Data presented as overall mean values ±1 SD, with range of mean values for duplicate chambers in parentheses. Asterisks indicate values significantly less than the controls, according to ANOVA and Dunnett's procedure (one-tailed): *$p = 0.05$, **$p = 0.01$.
[b]Based on 50 2- to 3-day-old embryos initially exposed per duplicate.
[c]Based on number of fry present after thinning to a maximum of 20 per chamber at 14 d and that survived for 32 d of exposure.

Table 3c. Survival length and weight of smallmouth bass after exposure to ammonia at pH 7.83[a]

Response	Total N: / NH_3:	Ammonia concentration (mg/L)						
		18.3 ± 1.59 0.760 ± 0.125	11.7 ± 1.33 0.472 ± 0.118	6.92 ± 1.04 0.274 ± 0.0933	3.75 ± 0.435 0.151 ± 0.0298	2.37 ± 0.175 0.0881 ± 0.0229	1.88 ± 0.363 0.0788 ± 0.0300	Control
Survival of 7-d-old sac fry (%)[b]		85 (84–86)	87 (82–92)	81 (76–86)	88 (86–90)	76 (70–82)	78 (76–80)	73 (68–78)
Survival of 14-d-old fry (%)[b]		38 (32–44)	46 (40–52)	51 (46–56)	64 (62–66)	56 (54–58)	64 (52–76)	62 (62–62)
Survival of 32-d-old fish (%)[c]		61** (60–62)	92 (90–95)	92 (85–100)	98 (95–100)	90 (90–90)	92 (90–95)	92 (90–95)
Mean total length of 32-d-old fish (mm)		20.5 ± 2.76** (19.9–21.1)	25.4 ± 1.78* (25.3–25.5)	25.9 ± 1.54 (25.9–26.0)	26.0 ± 1.39 (25.8–26.1)	26.6 ± 1.25 (26.6–26.7)	26.2 ± 1.36 (25.9–26.4)	26.4 ± 1.30 (26.1–26.8)
Mean wet weight of 32-d-old fish (mg)		118 ± 48.4** (104–134)	191 ± 39.2 (185–197)	200 ± 34.9 (196–204)	200 ± 34.2 (190–210)	215 ± 25.9 (212–218)	201 ± 28.8 (191–211)	206 ± 24.7 (205–208)

Table 3d. Survival, length and weight of smallmouth bass after exposure to ammonia at pH 8.68[a]

Response	Total N: / NH_3:	Ammonia concentration (mg/L)						
		4.96 ± 0.354 1.45 ± 0.220	3.16 ± 0.226 0.865 ± 0.124	1.82 ± 0.161 0.433 ± 0.0833	1.07 ± 0.173 0.240 ± 0.0482	0.682 ± 0.173 0.135 ± 0.0552	0.347 ± 0.156 0.0695 ± 0.0320	Control
Survival of 7-d-old sac fry (%)[b]		0** (0–0)	58 (54–62)	88 (84–92)	85 (74–96)	76 (72–80)	87 (86–88)	78 (72–84)
Survival of 14-d-old fry (%)[b]		0** (0–0)	28** (24–32)	50 (50–50)	63 (50–76)	54 (50–58)	68 (68–68)	67 (58–76)
Survival of 32-d-old fish (%)[c]		–	75** (69–83)	90 (90–90)	92 (90–95)	100 (100–100)	92 (90–95)	98 (95–100)
Mean total length of 32-d-old fish (mm)		–	22.4 ± 3.23** (22.1–22.8)	25.8 ± 1.26 (25.6–26.1)	26.2 ± 1.75 (25.9–26.5)	26.1 ± 1.68 (25.4–26.8)	25.6 ± 1.30 (25.2–26.1)	26.2 ± 1.18 (26.1–26.2)
Mean wet weight of 32-d-old fish (mg)		–	156 ± 65.3** (147–165)	195 ± 28.0 (190–200)	204 ± 35.8 (196–211)	209 ± 31.4 (199–218)	199 ± 28.8 (190–209)	202 ± 20.9 (192–211)

[a]Data presented as overall mean values ±1 SD, with range of mean values for duplicate chambers in parentheses. Asterisks indicate values significantly less than the controls, according to ANOVA and Dunnett's procedure (one-tailed): $*p = 0.05$, $**p = 0.01$.
[b]Based on 50 2- to 3-day-old embryos initially exposed per duplicate.
[c]Based on number of fry present after thinning to a maximum of 20 per chamber at 14 d and that survived for 32 d of exposure.

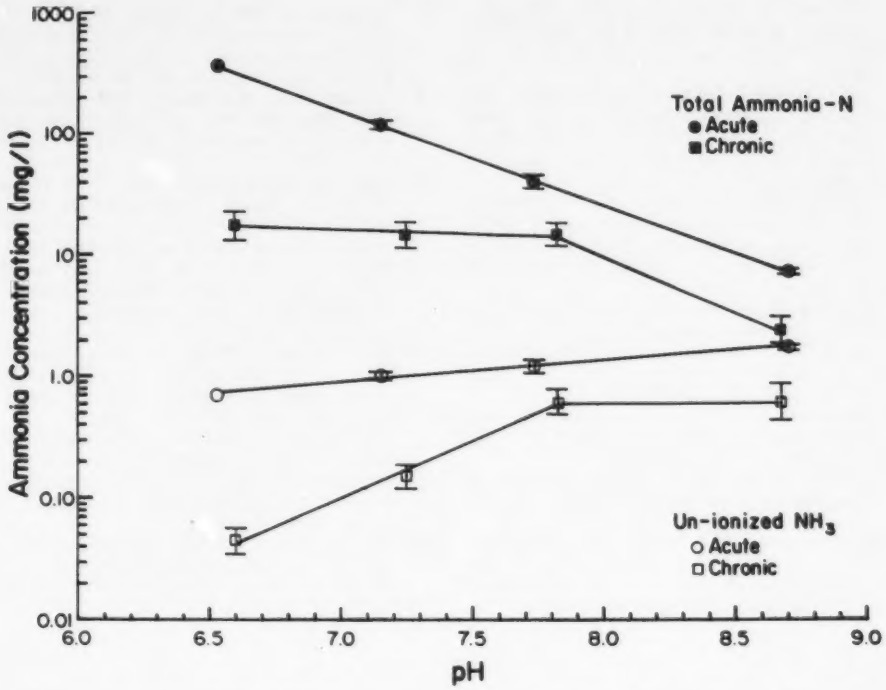

Fig. 1. Comparison of acute (96-h LC50 concentrations; circles with 95% confidence intervals indicated by error bars) and chronic (32-d no-observed-effect concentrations; squares with maximum no-effect and minimum effect concentrations indicated by limit bars) toxicity of ammonia to smallmouth bass within the pH range of 6.5–8.7.

REFERENCES

1. **Thurston, R.V., R.C. Russo** and **G.A. Vinogradov**. 1981. Ammonia toxicity to fishes: Effects of pH on the toxicity of un-ionized ammonia species. *Environ. Sci. Technol.* **15**:837–840.
2. **Emerson, K., R.C. Russo, R.E. Lund** and **R.V. Thurston**. 1975. Aqueous ammonia equilibrium calculations: Effect of pH and temperature. *J. Fish. Res. Board Can.* **32**:2379–2383.
3. **U.S. Environmental Protection Agency**. 1977. Ammonia. In *Quality Criteria for Water*. Office of Water and Hazardous Materials, U.S. Environmental Protection Agency, Washington, DC, pp. 10–13.
4. **Willingham, W.T., J.E. Colt, J.A. Fava, B.A. Hillaby, C.L. Ho, M. Katz, R.C. Russo, D.L. Swanson** and **R.V. Thurston**. 1979. Ammonia. In R.V. Thurston, R.C. Russo, C.M. Fetterolf, Jr., T.A. Edsall and Y.M. Barber, Jr., eds., *A Review of the EPA Red Book: Quality Criteria for Water*. Water Quality Section, American Fisheries Society, Bethesda, MD, pp. 6–18.
5. **Alabaster, J.S.** and **R. Lloyd**. 1982. *Water Quality Criteria for Freshwater Fish*, 2nd ed. Food and Agriculture Organization of the United Nations, Butterworth Scientific, Boston, MA.
6. **Roseboom, D.P.** and **D.L. Richey**. 1977. Acute toxicity of residual chlorine and ammonia to some native Illinois fishes. Illinois State Water Survey Report of Investigation 85, Urbana, IL.
7. **McCormick, J.H., S.J. Broderius** and **J.T. Fiandt**. 1984. Toxicity of ammonia to early life stages of the green sunfish, *Lepomis cyanellus*. *Environ. Pollut.* (Series A). **36**:147–163.
8. **Biesinger, K.E.** and **G.M. Christensen**. 1972. Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*. *J. Fish. Res. Board Can.* **27**:1691–1700.
9. **Benoit, D.A., V.R. Mattson** and **D.L. Olson**. 1982. A continuous-flow mini-diluter system for toxicity testing. *Water Res.* **16**:457–464.
10. **Sprague, J.B**. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. *Water Res.* **3**:793–821.
11. **Mount, D.I**. 1968. Chronic toxicity of copper to fathead minnows. *Water Res.* **2**:215–223.
12. **Drummond, R.A.** and **W.F. Dawson**. 1970. An inexpensive method for simulating diel patterns of lighting in the laboratory. *Trans. Am. Fish. Soc.* **99**:434–435.
13. **American Public Health Association, American Water Works Association** and **Water Pollution Control Federation**. 1980. *Standard Methods for the Examination of Water and Wastewater*, 15th ed. Washington, DC.
14. **Thurston, R.V., R.C. Russo** and **K. Emerson**.

1979. Aqueous ammonia equilibrium—Tabulation of percent un-ionized ammonia. EPA-600/3-79-091. U.S. EPA, Ecological Research Series, Duluth, MN.

15. **American Society for Testing and Materials**. 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Annual Book of ASTM Standards. E729-80. Philadelphia, PA.
16. **Hamilton, M.A., R.C. Russo** and **R.V. Thurston**. 1977. Trimmed Spearman-Karber method for estimating medium lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.* **11**:714–719. Correction **12**:417 (1978)..
17. **American Society for Testing and Materials**. 1983. Proposed standard practice for conducting toxicity tests with the early life-stages of fishes. ASTM E-47.01, Draft No. 5. American Society for Testing and Materials. Philadelphia, PA.
18. **Balon, E.K**. 1975. Terminology of intervals in fish development. *J. Fish. Res. Board Can.* **32**: 1663–1670.
19. **Steel, R.G.D.** and **J.H. Torrie**. 1960. *Principles and Procedures of Statistics*. McGraw-Hill Book Co., Inc., New York, NY.
20. **Konemann, H.** and **A. Musch**. 1981. Quantitative structure-activity relationships in fish toxicity studies. Part 2: The influence of pH on the QSAR of chlorophenols. *Toxicology* **19**:223–228.
21. **Lloyd, R.** and **D.W.M. Herbert**. 1960. The influence of carbon dioxide on the toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdneri* Richardson). *Ann. Appl. Biol.* **48**:399–404.
22. **Szumski, D.S., D.A. Barton, H.D. Putman** and **R.C. Polta**. 1982. Evaluation of EPA un-ionized ammonia toxicity criteria. *J. Water Pollut. Control Fed.* **54**:281–291.
23. **Broderius, S.J., L.L. Smith, Jr.,** and **D.T. Lind**. 1977. Relative toxicity of free cyanide and dissolved sulfide forms to the fathead minnow *Pimephales promelas*. *J. Fish. Res. Board Can.* **34**:2323–2332.
24. **Russo, R.C., R.V. Thurston** and **K. Emerson**. 1981. Acute toxicity of nitrite to rainbow trout (*Salmo gairdneri*): Effects of pH, nitrite species, and anion species. *Can. J. Fish. Aquat. Sci.* **38**:387–393..
25. **Rice, S.D.** and **J.E. Bailey**. 1980. Survival, size, and emergence of pink salmon, *Oncorhynchus gorbuscha*, alevins after short- and long-term exposures to ammonia. *Fish. Bull.* **78**:641–648.
26. **Calamari, D., R. Marchetti** and **G. Vailati**. 1981. Effects of long-term exposure to ammonia on the developmental stages of rainbow trout (*Salmo gairdneri* Richardson). *Rapp. P.-V. Reun. Cons. Int. Explor. Mer.* **178**:81–86.
27. **Robinson-Wilson, E.F.** and **W.K. Seim**. 1975. The lethal and sub-lethal effects of a zirconium process effluent on juvenile salmonids. *Water Res. Bull.* **11**:975–986.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.